PATENT SPECIFICATION

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(19)

(54) ANTIBACTERIAL AGENT AND A PROCESS FOR THE PREPARATION THEREOF

(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the Laws of the State of Delaware, United States of America, of 345 Park Avenue, New York, State of New York, 10022, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a new synthetic compound of value as an antibacterial agent, as a nutritional supplement in animal feed, as an agent for the treatment of mastitis in cattle, and as a therapeutic agent in poultry and animals, including man, in the treatment, particularly by oral administration, of infectious diseases caused by many Gram-positive and Gram-negative bacteria.

Cephalothin and cephaloridine are well-known antibacterial agents; See U.S. Patents 3,218,318; 3,449,338 and 3,498,979. The patent literature also contains considerable data on cephaloglycin and cephalexin; see U.S. Patents 3,303,193; 3,422,103; 3,364,212 and 3,507,861 and Great Britain 985,747; 1,054,806 and 1,174,335 and Belgium 696,026 and South Africa 67/1260. Newer cephalosporins include cefazolin and cephapirin; see U.S. Patent 3,516,997 [and also Netherlands 68/05179 and South Africa 68/4513] and U.S. Patent 3,422,100.

The literature on cephalosporins has been reviewed for example, by E. P. Abraham, *Pharmacol, Rev. 14,* 473—500 (1962), by I. M. Rollo, *Ann. Rev. Pharmacol, 6,* 218—221 (1966) by E. P. Abraham, *Quart. Rev.* (London) *21* 231 (1967) by E. Van Heyningen Advan. Drug Res., 4, 1—70 (1967), by G. T. Stewart, The Penicillin Group of Drugs, Elsevier Publishing Company, New York, N.Y. (1965) at pages 185—192 and briefly in *Annual Reports in Medicinal Chemistry,* Academic Press, Inc., 111 Fifth Avenue, New York, New York, 10003, by L. C. Cheney on pages 96 and 97 (1967) and by K. Gerzon and R. B. Morin on pages 90—93 (1968) and K. Gerzon on pages 78—80 (1969). New cephalosporins are frequently reported at the annual Interscience Conference on Antimicrobial Agents and Chemotherapy as illustrated by Sassiver et al., *Antimicrobial Agents and Chemotherapy*—1968, American Society for Microbiology, Bethesda, Maryland, pages 101—114 (1969) and by Nishida et al., *ibid,* 236—243 (1970).

7-phenylacetamidocephalosporanic acid has also been named N-phenylacetyl derivative of 7—ACA, cephaloram, PACA and apparently phenosporin. Publications in the scientific literature on the preparation and/or properties of this compound, with or without substituents in the benzene ring, and corresponding compounds in which the 3-acetoxy-methyl group has been replaced by methyl, hydroxymethyl and/or pyridiniummethyl include the following: Chauvette, R.R., et al. "Chemistry of Cephalosporin Antibiotics II.

Preparation of a New Class of Antibiotics and the Relation of Structure To Activity", *Journal of the American Chemical Society, 84,* 3401—3402 (1962). Chauvette, R.R. et al. "Structure-Activity of Relationships Among 7-acylamidocephalosporanic Acids", *Antimicrobial Agents and Chemotherapy—1962,* 687—694.

Cocker, J. D., et al., "Cephalosporanic Acids. Part II. Displacement of the Acetoxy-group by Nucleophiles", *Journal of the Chemical Society*, 5015—5031 (1965).
Cocker, J. D. et al., "Cephalosporanic Acids. Part IV. 7-Acylamidoceph-2-em-4-carboxylic Acids", *Journal of the Chemical Society*, 1142—1151 (1966).
Culp, H. W., et al., "Metabolism and Absorption of 7-(Phenylacetamido-1-C^{14})-Cephalosporanic Acid", *Antimicrobial Agents and Chemotherapy*—1963, 243—246.
Jago, M., "Antibacterial Activity of Some Derivatives of 7-Aminocephalosporanic acid Against *Staphylococcus Aureus* and Synergism Between these and Other Antibiotics", *Brit. J. Pharmacol.*, *22*, 22—33 (1964).
Loder, B., et al., "The Cephalosporin C Nucleus (7-Aminocephalosporanic Acid) and some of its Derivatives", *Biochemical Journal*, *79*, 408—416 (1961)
Nishida, M., et al., "Studies on Microbial Degradation of Cephalosporin C Derivatives. II", *The Journal of Antibiotics*, *21*, 375—378 (1968).
Nishida, M., et al., "Studies of Microbial Degradation of Cephalosporin C Derivatives I.:, *The Journal of Antibiotics*, *21*, 165—169 (1968).
Spencer, J. L., et al., "Chemistry of Cephalosporin Antibiotics VIII. Synthesis and Structure-Activity Relationships of Cephaloridine Analogues", *Antimicrobial Agents and Chemotherapy—1966*, 573—580.
Stedman, R. J. et al., "7-Aminodesacetoxycephalosporanic Acid and its Derivatives", J. Med. Chem., *7(1)*, 117—119 (1964).
Sullivan, H. R., et al., "Metabolism of Oral Cephalothin and Related Cephalosporins in the Rat", *Biochemical Journal*, *102*, 976—982 (1967).
Vymola, F., et al., "The Classification and Characteristics of Cephalosporin Antibiotics I. Systematic Study of the Quantitative Sensitivity of Some Pathogenic Microorganisms to Cephaloridine", *Journal of Hygiene, Epidemiology, Microbiology and Immunology*, *10*, 180—189 (1966).

Many other 7-acyl derivatives of 7-aminocephalosporanic acid have been reported in the patent literature including 7-[4-(α-aminoalkyl)phenylacetamido]cephalosporanic acids (U.S. Patent 3,382,241), 7-[(*p*-aminophenylthio)acetamido]cephalosporanic acid (U.S. patent 3,422,100), 7-halophenylthioacetamido)cephalosporanic acids (U.S. patent 3,335,136) and the nearly unlimited number of variations of such compounds encompassed by the generic formulae (and often not otherwise described) of such patents as Netherlands 69/02013. 7-(*p*-Aminophenylacetamido)-cephalosporanic acid is disclosed in U.S. patent 3,422,103 as is the corresponding N-trityl derivative; see also Japan 2712/67.

U.S. patent 3,219,662 includes claims to compounds of the structure

$$R—CH_2—CO—ACA$$

in which R is phenyl, nitrophenyl (especially para-nitro), chlorophenyl, alkylphenyl and alkoxyphenyl and the corresponding phenoxy and substituted compounds and for all of those the corresponding compounds in which the 3-acetoxymethyl group has been replaced by a 3-pyridiniummethyl group. A more extensive group of such compounds, including the series in which R is phenylthio and also the compounds in which R is benzyl [i.e., 7-(β-phenylpropionamido)cephalosporanic acid], alkoxybenzyl, alkanoyloxybenzyl, aminobenzyl, etc. are disclosed, at least generically, for use as starting materials in Great Britain 1,012,943 and 1,153,421 and see also Great Britain 1,001,478 and U.S. 3,280,118. Additional 7-phenylacetamidocephalosporanic acids having substituents on the benzene ring including hydroxy and amino are disclosed as starting materials in Great Britain 1,082,943 and 1,082,962.

U.S. Patent 3,341,531 describes the 7-(*o*-, *m*- and *p*-carboxamidomethylphenylacetamido)cephalosporanic acids and their betaines. A variety of 7-(halo-, dihalo, nitro- and halonitro-phenylacetamido)cephalosporanic acids are named as starting materials for reaction with certain nucleophiles in U.S. patent 3,431,259. Additional 7-(phenylacetamido)cephalosporanic acids having various substituents on the benzene ring are disclosed in Japan 2712/67, Japan 26105/69, Great Britain 1,178,471 (see Netherlands 67/00906) and Japan 25785/69.

Replacement of the 3-acetoxy group of a cephalosporin by various heterocyclic thiols has been disclosed.

a) in South Africa 70/2290 [see also Netherlands 70/05519] where the side-chains were, for example, 7-α-aminophenylacetamido and typical heterocyclic thiols were 2-methyl-1,3,4-thiadiazole-5-thiol and 1-methyl-1,2,3,4-tetrazole-5-thiol, and

b) in U.S. 3,516,997 where the sidechains at the 7-position had structures such as R^3—(alk)*m*—CO—NH— and R^3—S—(alk)*m*—CO—NH— in which R^3 was one of many aromatic heterocycles and the numerous heterocyclic thiols at the 3-position included, for example, 1-methyl-tetrazole-5-thiol and 2-methyl-1,3,4-thiadiazol-5-thiol, and

c) in U.S. patent 3,563,983.

U.S. patent 3,492,297 includes 7-(*p*-guanidinophenylacetamido)cephalosporanic acid and its betaine.

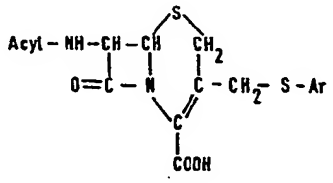
In cephaloridine the 3-acetoxy group of cephalothin was replaced by a pyridinium group as described, for example, in U.S. patents 3,449,338 and 3,498,979.

The preparation of various 7-[α-amino-arylacetamido]-cephalosporanic acids and the corresponding desacetoxy compounds in which aryl represents unsubstituted or substituted phenyl or 2- or 3-thienyl is described, for example, in British Specifications 985,747, 1,017,624, 1,054,806 and 1,123,333, in Belgian patent 696,026, in U.S. patents 3,311,621, 3,352,858, 3,489,750, 3,489,751, 3,489,752 and 3,518,260, in Japanese patent 16871/66, by Spencer et al., *J. Med. Chem., 9(5)*, 746—750 (1966) and by Kurita et al., *J. Antibiotics* (Tokyo) (A) *19*, 243—249 (1966) and see also U.S. patent 3,485,819.

Netherlands patents 68/11676 and 68/12382 and U.S. patents 3,489,750 and 3,489,751 and 3,489,752 disclose ring-substituted cephaloglycins.

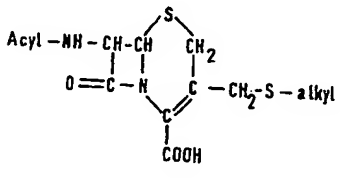
Various 7-[α-amino-arylacetamido]cephalosporins in which one hydrogen of the α-amino group is replaced by a carbonyl group which is attached in turn to another moiety have been reported. The earliest were the cephaloglycin and cephalexin precursors in which use was made of a common peptide blocking group such as carbobenzyloxy as illustrated by U.S. patent 3,364,212, Belgian patent 675,298, South African patent 67/1260 and Belgian patent 696,026. Related compounds include those of U.S. patents 3,303,193 and 3,311,621 and 3,518,260.

Various cephalosporins, including cephalosporin C on occasion but not cephaloglycin, have been reacted with nucleophilic, aromatic mercaptans to produce compounds having the structure



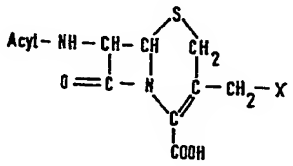
In U.S. Patent 3,278,531 Ar is phenyl or certain substituted phenyls or certain aromatic heterocyclic rings named, for example, in column 5. Similar nucleophiles, e.g. 2-mercaptopyrimidines, are disclosed in U.S. 3,261,832 and Great Britain 1,101,422 and U.S. 3,479,350 and U.S. 3,502,665, all issued to Glaxo. A parallel disclosure is found in Great Britain 1,109,525 to Ciba, e.g. in definition "h" for R_3. Additional nucleophiles of this type were disclosed by Fujisawa in Belgium 714,518, (Netherlands 68/06129 and South Africa 2695/68), in Canada 818,501, in Great Britain 1,187,323; U.S. 3,530,123; Netherlands 67/14888) and especially in U.S. 3,516,997 (Netherlands 68/05179) which includes the compound named cefazolin, which has a tetrazolylacetyl sidechain on the 7-amino group and a 5-methyl-thiadiazolylthiomethyl group at the 3-position and is described at some length in the scientific literature, e.g. in Antimicrobial Agents and Chemotherapy—1969, American Society for Microbiology, Bethesda, Maryland at pages 236—243 and in J. Antibiotics (Japan) *23(3)*, 131—148 (1970).

Various cephalosporins having the structure

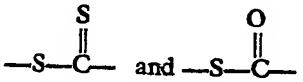


in which acyl represents various sidechains including α-aminophenylacetyl have been described in some of the above and by Glaxo in Belgium 734,532 and in Belgium 734,533.

Cephalosporins having the structure



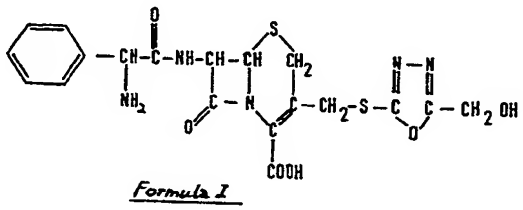
where X includes



are disclosed in some of the above and in U.S. 3,239,515, 3,239,516, 3,243,435, 3,258,461, 3,431,259 and 3,446,803.

Related publications in the scientific literature include J. Med. Chem. *8*, 174—181 (1965) and J. Chem. Soc. (London) 1595—1605 (1965), 5015—5031 (1965) and 1959—1963 (1967).

According to the present invention there is provided a compound having the formula

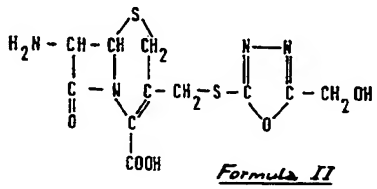


Formula I

This compound is amphoteric and exists primarily in the zwitterion form. Preferably the compound has the D configuration in the sidechain.

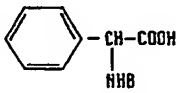
The invention also includes non-toxic, pharmaceutically acceptable salts of the above compound. Such salts include the non-toxic carboxylic acid salts thereof, including non-toxic metallic salts such as sodium, potassium, calcium and aluminium, the ammonium salt and substituted ammonium salts, e.g. salts of such non-toxic amines as trialkylamines, including triethylamine, procaine, dibenzylamine, N-benzylbetaphenethylamine, 1-ephenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylethylenediamine, N-(lower C_1—C_8)alkylpiperidine, e.g., N-ethylpiperidine and other amines which have been used to form salts with benzylpenicillin and the non-toxic addition salts thereof (i.e., the amine salts) including the mineral acid addition salts such as the hydrochloride, hydrobromide, hydroiodide, sulfate, sulfamate and phosphate and the organic acid addition salts such as the maleate, acetate, citrate, oxalate, succinate, benzoate, tartrate, fumarate, malate, mandelate and ascorbate.

The invention also provides a process for the preparation of a compound having the above Formula I or a non-toxic, pharmaceutically acceptable salt thereof, which process comprises reacting a compound having the formula



Formula II

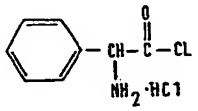
or a salt or easily hydrolyzed ester thereof with an acid having the formula



wherein B represents a blocking group, or with a functional equivalent of the acid as an acylating agent for the primary amino group, and thereafter, if necessary, removing any blocking group.

Suitable easily hydrolyzed esters include those of U.S. Patent 3,284,451 and any of the silyl esters described in U.S. Patent 3,249,622 for use with 6-aminopenicillanic acid and used in Great Britain 1,073,530.

The blocking group B may be of the type used either in peptide synthesis or in any of the numerous syntheses of α-aminobenzylpenicillin from 2-phenylglycine. Particularly valuable blocking groups are a proton, as in the compound of the formula



or a β-diketone as in Great Britain 1,123,333, e.g. methyl acetoacetate in which case the acid containing the blocked amino group is preferably converted to a mixed anhydride as with ethyl chloroformate, before reaction with compound II or a salt thereof to form the desired product I after acid cleavage.

Examples of suitable blocking groups and the methods used for their removal are:

The *t*-butoxy-carbonyl group which is removed by treatment with formic acid,

The carbobenzyloxy group which is removed by catalytic hydrogenation,

The 2-hydroxy-1-naphthcarbonyl group which is removed by acid hydrolysis, and

The trichloroethoxycarbonyl group which is removed by treatment with zinc dust in glacial acetic acid.

With respect to the acid to be used to couple with compound II, functional equivalents include the corresponding acid anhydrides, including mixed anhydrides and particularly the mixed anhydrides prepared from stronger acids such as the lower aliphatic monoesters of carbonic acid, or alkyl and aryl sulfonic acids and of more hindered acids such as diphenylacetic acid. In addition, an acid azide or an active ester or thioester (e.g., with *p*-nitrophenol, 2,4-dinitrophenol, thiophenol, thioacetic acid) may be used or the free acid itself may be coupled with compound II after first reacting said free acid with N,N'-dimethylchloroforminium chloride [cf. Great Britain 1,008,170 and Novak and Weichet, *Experientia XXI*, *6* 360, (1965)] or by the use of enzymes or of an N,N'-carbonyldiimidazole or an N,N'-carbonylditriazole [cf. South African Patent Specification 63/2684] or a carbodiimide reagent [especially N,N'-dicyclohexylcarbodiimide, N,N'-diisopropylcarbodiimide or N-cyclohexyl-N'-(2-morpholinoethyl)carbodiimide; cf. Sheehan and Hess, *J. Amer. Chem. Soc.*, *77*, 1067 (1955)], or of alkylylamine reagent [cf. R. Buijle and H. G. Viehe, *Angew, Chem. International* Edition 3, 582 (1964)], or of a ketenimine reagent [cf. C. L. Stevens and M. E. Mond, *J. Amer. Chem. Soc., 80* (4065)] or of an isoxazolium salt reagent [cf. R. B. Woodward, R. A. Olofson and H. Mayer, *J. Amer. Chem. Soc., 83*, 1010 (1961)]. Another equivalent of the acid chloride is a corresponding azolide, i.e. an amide of the corresponding acid whose amide nitrogen is a member of an quasiaromatic fivemembered ring containing at least two nitrogen atoms, i.e., imidazole, pyrazole, the triazoles, benzimidazole, benzotriazole and their substituted derivatives. As an example of the general method for the preparation of an azolide, N,N'-carbonyldiimidazole is reacted with a carboxylic acid in equimolar proportions at room temperature in tetrahydrofuran, chloroform, dimethylformamide or a similar inert solvent to form the carboxylic acid imidazolide in practically quantitative yield with liberation of carbon dioxide and one mole of imidazole. Dicarboxylic acids yield dimidazolide. The by-product, imidazole, precipitates and may be separated and the imidazolide isolated, but this is not essential. The methods for carrying out these reactions to produce a cephalosporin and the methods used to isolate the cephalosporin so produced are well known in the art.

In the treatment of bacterial infections in man, the compounds of this invention are administered orally or parenterally, in accordance with conventional procedures for antibiotic administration, in an amount of from about 5 to 200 mg./kg./day and preferably about 5 to 20 mg./kg./day in divided dosage, e.g., three to four times a day. They are administered in dosage units containing, for example, 125 or 250 or 500 mg. of active ingredient with suitable physiologically acceptable carriers or excipients. The dosage units are in the form of liquid preparations such as solutions or suspensions or as solids in tablets or capsules.

Thus, the invention also includes a pharmaceutical composition, which composition comprises a compound of the above Formula I or a non-toxic, pharmaceutically acceptable salt thereof and a physiologically acceptable carrier or excipient.

The invention further includes a method of treating non-human mammals for diseases caused by Gram-positive or Gram-negative bacteria such as mastitis in cattle, which method comprises administering to the mammal an effective amount of a compound of the above Formula I, a non-toxic, pharmaceutically acceptable salt thereof or a composition as defined above.

Exactly 200 g. of 7-aminocephalosporanic acid (7—ACA) was suspended in 500 ml. of acetone and a solution of 240 g. of *p*-toluenesulfonic acid in 500 ml. of acetone was added in one charge. After stirring for five minutes, at room temperature, the mixture was filtered through diatomaceous earth ("Super Cel") and the bed washed with 150 ml. of acetone (the insoluble matter weighed about 30 g.). Then 80 ml. of water was added to the filtrate and, while stirring, the *p*-toluene-sulfonate salt crystallized out after scratching on the inside of the flask with a glass rod. The suspension was stirred in an ice-salt bath for thirty minutes and filtered cold. It was washed with 2×200 ml. of cold acetone (0° C.) and air dried; yield 250 g. of salt. This *p*-toluene-sulfonate salt of 7—ACA was stirred in 2 liters of methanol and the insoluble matter filtered through "Super Cel". The filtrate was placed in a five liter 3 neck flask and 2 liters of water were added. Then the pH was adjusted to 4 by the addition of concentrated ammonium hydroxide with cooling and the suspension stirred for one hour at 0° C. The product was collected by filtration and washed with 2×100 ml. H_2O (0° C.) and 3×1 liter acetone (room temperature). After air drying, the yield of 7—ACA was 145 g. ("Super Cel" is a Registered Trade Mark).

Reference: Glaxo, British Patent 1,104,938 (1968).

The following examples are given in illustration of, but not in limitation of, the present invention. All temperatures are in degrees Centigrade. 7-Aminocephalosporanic acid is abbreviated as 7—ACA and methyl isobutyl ketone as MIBK. "Skellysolve B" is a petroleum ether fraction of B.P. 60—68° C. consisting essentially of *n*-hexane.

Example 1.

Sodium D-α-[1-carbomethoxypropen-2-yl)-amino]-phenylacetate

Lit. ref. E. Dane, F. Oreis, P. Konrad, T. Dockner, *Angew. Chem. Intern. Ed. Engl. 1,* 658 (1962); E. Dane and T. Dockner *Angew. Chem. 76,* 342 (1964); Spencer, Flynn, Roeske, Sin and Chauvette, *J. Med. Chem., 9,* 746—50 (1966); U.S. Patent 3,496,171 (Lilly).

To a well stirred mixture of 40 g. (1 mole) of NaOH in 40 ml. of H_2O and one liter of benzene was added 151.6 g. (1 mole) of D—(−)-phenylglycine. The mixture was held at about 55° C. for 30 minutes and then with vigorous stirring 116 g. (1 mole) of methyl acetoacetate was added and the mixture stirred and heated at reflux until no more water was collected in the Dean Stark trap. Next one liter of acetone was added with the heat removed and then the slurry was cooled and stirred 30 minutes in an ice-salt bath. The product was collected by filtration, washed well with copious amounts of acetone and air dried. Yield was 191 g., dec. pt. 252° C., $[\alpha]_D^{22°C.}$ + 207°.

(C=1%, H_2O).

Hydroxyacethydrazide

Ethyl glycolate (10.41 g., 0.10 mole) was dissolved in 15 ml. of absolute ethanol and treated dropwise with 7.5 ml. (0.10 mole) of 85% hydrazine hydrate. The mixture was heated at reflux for 1 hour and then cooled. Evaporation under reduced pressure gave a liquid residue which solidified on treatment with a small volume of ethanol. This material (7.2 g., m.p. 87—92° C.) was crystallized from ethanol giving 6.6 g. (73%) of hydroxyacethydrazide with m.p. 90—93° C.

2-Mercapto-5-(Hydroxymethyl)-1,3,4-Oxadiazole Potassium Salt.

Hydroxyacethydrazide (18.0 g., 0.20 mole) and potassium hydroxide (11.2 g., 0.20 mole) were treated with 400 ml. of absolute ethanol containing 30 ml. of dimethylsulfoxide. This solution was then stirred and treated with 60 ml. of carbon disulfide resulting in the formation of a yellow crystalline solid. The stirred mixture was heated at reflux and after 36 hours evolution of hydrogen sulfide had ceased.

Cooling and filtration gave 21.6 g. of the crystalline product with m.p. 178—80° C. Evaporation of the filtrate and crystallization of the residue from ethanol gave a further 2.91 g. (72%).

Further crystallizations raised the m.p. to 179.5—181° C., Calculated for $C_3H_3KN_2O_2S$: C, 21.17; H, 1.78; N, 16.46. Found: C, 21.28; H, 1.77; N, 16.58.

7 - Amino - 3 - [S - (5 - hydroxymethyl - 1,3,4 - oxadiazole - 2 - yl) - thiomethyl] - 3 - cephem - 4 - carboxylic acid.

A mixture of 17.0 g. (0.1 mole) of 2-mercapto-5-hydroxymethyl-1,3,4-oxadiazole potassium salt, 27.2 g. (0.1 mole) of 7—ACA, 8.4 g. (0.1 mole) of $NaHCO_3$ in 500 ml. of 0.1 *M* phosphate buffer, pH 6.4, was heated at 55° C. for five hours. The resulting solution was cooled to 20° C. and acidified to pH 5.5 with 40% H_3PO_4. After stirring 15 min. the precipitate was collected by filtration, washed with 50 ml. of cold water and then 200 ml. of acetone and air dried. After vacuum drying over P_2O_5 there was obtained 13.5 g. of 7-amino-3-[S-(5-hydroxymethyl-1,3,4-oxadiazole-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid, dec. pt>100° C. (indefinite). The ir and nmr spectra were entirely consistent with the desired structure. Anal. Calcd. for $C_{11}H_{12}N_4O_5S_2$: C, 38.37; H, 3.52; N, 16.28. Found: C, 36.93; H, 3.78; N, 16.48.

7 - [D - α - Amino - α - phenylacetamido] - 3 - [S - (5 - hydroxymethyl - 1,3,4-oxadiazole - 2 - yl) - thiomethyl] - 3 - cephem - 4 - carboxylic acid.

To a stirred suspension of 8.13 g. (0.03 mole) of sodium D-α-[1-carbomethoxypropen-2-yl)amino]-phenyl acetate in 100 ml. of acetonitrile and 0.1 ml. of N,N-dimethylbenzylamine, cooled to —10° C., was added 3.53 g. (0.033 mole) of ethyl chloroformate. After 20 min. at —10° C. a solution of 10.33 g. (0.03 mole) of 7-amino - 3 - [S - (5 - hydroxymethyl - 1,3,4 - oxadiazole - 2 - yl) - thiomethyl] - 3-cephem-4-carboxylic acid, 50 ml. water, 50 ml. of acetonitrile and 4.2 ml. (0.03 mole) of triethylamine, precooled to 0° C. was added all at once with vigorous stirring. The temperature was kept at 0° C. for 45 min. and then salt (NaCl) was added in excess to saturate the solution. This took 15 min. The organic (top) layer was separated and 40 ml. of water was added to it. The resulting solution was then concentrated at reduced pressure at 20° C. to a volume of about 50 ml. To this aqueous solution was added a solution of 100 ml. of methyl isobutyl ketone (MIBK) and 15 ml. of 90% formic acid and the mixture shaken for a few seconds and then stirred at 0° C. for three hours. The aqueous phase was then separated and the pH adjusted to 3.3 with solid $NaHCO_3$. Fresh MIBK (100 ml.) was added and the mixture cooled and stirred at 0° C. for 1 hr. The gum which separated was stirred with 60 ml. of 10% H_3PO_4 for 15 min. and filtered. The filtrate was stirred 10 min. with 1 g. of "Darko KB" decolourizing carbon, filtered again and the pH adjusted to 3.4 with solid $NaHCO_3$. (Darko is a Registered Trade Mark). The resulting slurry was cooled at 0° C. for 30 min. and the product, 7-[D-α-amino-α-phenylacetamido]-3-[S-(5-hydroxymethyl-1,3,4-oxadiazole-2-yl)-thiomethyl]-3-cephem-4-carboxylic acid was collected by filtration, washed with 2 ml. of ice cold water and air dried. Yield 210 mg. dec. a 210° C. A second crop, obtained by concentrating the filtrate, weighed 300 mg. This second crop had an ir and nmr spectra entirely consistent with the desired structure, but contained about 25% salts as a contaminate.

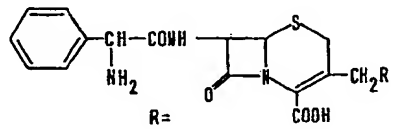
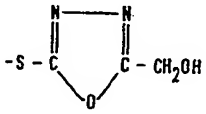
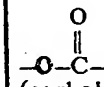
7 - [D - (α - Amino - α - phenylacetamido)] - 3 - [S - (5 - hydroxymethyl-1,3,4 - oxadiazol - 2 - yl)thiomethyl] - 3 - cephem - 4 - carboxylic acid (called New Compound) after solution in 5% $NaHCO_3$ (for Lot 01) or dimethylsulfoxide (for Lot 02) followed by dilution with Nutrient Broth was found to exhibit the following Minimum Inhibitory Concentrations (M.I.C.) in mcg./ml. versus the indicated microorganisms as determined by overnight incubation at 37° C. by Tube Dilution. Results with four old compounds are also given.

TABLE 1

Organism		MIC in mcg./ml. New Cpd. Lot 1	New Cpd. Lot 2	Cephalexin	Cephaloglycin	Cephalothin	Cephaloridine
*D. pneumoniae**	A9585	.08	.02	.16	.04	.01	.004
Str. pyogenes+5%serum*	A9604	.08	.04	.3	.04	.08	.008
S. aureus Smith	A9537	2.5	2.5	1.3	.6	.16	.03
S-aureus Smith+50% serum	A9537	>1.3	>1.3	2.5	1.3	.3	.03
S. aureus BX1633/2 at 10^{-3} dil'n	A9606	2.5	2.5	10	1.3	.3	.16
S. aureus BX1633-2 at 10^{-2} dil'n	A9606	8	4	8	1.3	.6	4
S. aureus meth.–resistant	A15097	63	16	63	8	>2.5	5
Sal. enteritidis	A9531	2	.5	4	.3	.3	.6
E. coli Juhl	A15119	8	2	8	.5	16	2
E. coli	A9675	32	8	32	2	63	8
K. pneumoniae	A9977	4	1	4	.3	2	1
K. pneumoniae	A15130	32	8	16	1	16	4
Pr. mirabilis	A9900	8	2	4	.6	1	1
Pr. morganii	A15153	125	63	>125	63	>125	>125
P. aeruginosa	A9843A	>125	>125	>125	>125	>125	>125
Ser. marcescens	A20019	>125	>125	>125	>125	>125	>125

*50% Nutrient Broth–45% Antibiotic Assay Broth.

Blood levels in the mouse after oral administration were determined with the following results:

	mgm./Kg. Dose	Blood level in mcg./ml. Hours after administration 0.5	1	2	3.5
	100	7.4	5.9	2.2	0.8
H (cephalexin monohydrate)	100	45	24.9	7.5	3.5
 (cephaloglycin dihydrate)	100	1.64	2.4	1.6	0.8

Example 2.

Sodium 7 - [D - (α - amino - α - phenylacetamido] - 3 - [S - (5 - hydroxymethyl-1,3,4 - oxadiazol - 2 - yl)thiomethyl] - 3 - cephem - 4 - carboxylate.

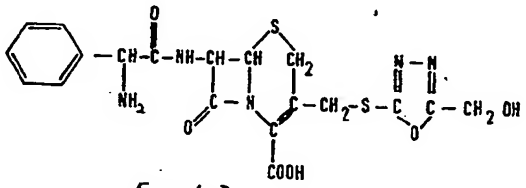
To a stirred aqueous suspension of the zwitterionic form of 7-[D-(α-amino-α-phenylacetamido)] - 3 - [S - (5 - hydroxymethyl - 1,3,4 - oxadiazol - 2 - yl)-thiomethyl]-3-cephem-4-carboxylic acid (0.8 mmole) is added 1 *N* aqueous sodium hydroxide at room temperature until a clear solution (pH 10.8 is obtained. This solution is immediately freeze-dried to give impure, solid sodium 7-[D-(α-amino-α-phenylacetamido)] - 3 - [S - (5 - hydroxymethyl - 1,3,4 - oxadiazol - 2 - yl)thiomethyl]-3-cephem-4-carboxylate.

Example 3.

A suspension of the zwitterionic form of 7-[D-α-amino-α-phenylacetamido]-3-[S-(5-hydroxymethyl - 1,3,4 - oxadiazole - 2 - yl) - thiomethyl] - 3 - cephem - 4-carboxylic acid (0.361 g.) in 3 ml. of methanol is cooled in ice and treated with a few drops of concentrated hydrochloric acid until a clear solution is obtained. 7-[D-α-amino - α - phenylacetamido] - 3 - [S - (5 - hydroxymethyl] - 1,3,4 - oxadiazole - 2-yl)-thiomethyl]-3-cephem-4-carboxylic acid hydrochloride precipitates as a pale brown colored solid upon the addition of ether and is collected by filtration and dried *in vacuo* over P_2O_5.

WHAT WE CLAIM IS:—

1. A compound having the formula



Formula I

2. A compound as claimed in claim 1 having the D configuration in the side-chain.

3. A non-toxic, pharmaceutically acceptable salt of a compound as claimed in claim 1 or claim 2.

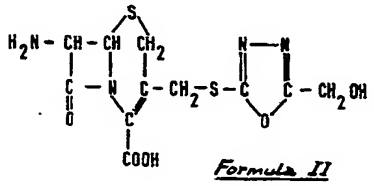
4. A salt as claimed in claim 3 with sodium, potassium, calcium, aluminium or ammonia.

5. A salt as claimed in claim 3 with triethylamine, procaine, dibenzylamine, N-benzyl-betaphenethylamine, 1-ephenamine, N,N'-dibenzylethylenediamine, dehydroabietylamine, N,N'-bis-dehydroabietylethylenediamine or N-ethylpiperidine.

6. A salt as claimed in claim 3 with hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfamic, phosphoric, maleic, acetic, citric, oxalic, succinic, benzoic, tartaric, fumaric, malic, mandelic or ascorbic acid.

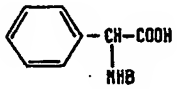
7. A compound as claimed in claim 1 or claim 2 in the zwitterion form.

8. A process for the preparation of a compound having the Formula I defined in claim 1 or a non-toxic, pharmaceutically acceptable salt thereof, which process comprises reacting a compound having the formula



Formula II

or a salt or easily hydrolysed ester thereof with an acid having the formula



wherein B represents a blocking group, or with a functional equivalent of the acid as an acylating agent for the primary amino group, and thereafter, if necessary, removing any blocking group.

9. A process as claimed in claim 8, wherein the blocking group is *t*-butoxycarbonyl and the blocking group is removed with formic acid.

10. A process as claimed in claim 8, wherein the blocking group is carbobenzyloxy and the blocking group is removed by catalytic hydrogenation.

11. A process as claimed in claim 8, wherein the blocking group is 2-hydroxy-1-napthcarbonyl and the blocking group is removed by acid hydrolysis.

12. A process as claimed in claim 8, wherein the blocking group is trichloroethoxycarbonyl and the blocking group is removed with zinc dust in glacial acetic acid.

13. A process as claimed in claim 8 substantially as hereinbefore described in any one of the specific Examples.

14. A compound of the Formula I defined in claim 1 or a non-toxic, pharmaceutically acceptable salt thereof when prepared by a process as claimed in any one of claims 8 to 13.

15. A pharmaceutical composition, which composition comprises a compound as claimed in any one of claims 1, 2, 7 or 14 or a salt as claimed in any one of claims 3 to 6 or 14 and a physiologically acceptable carrier or excipient.

16. A method of treating mammals non-human for diseases caused by Gram-positive or Gram-negative bacteria, which method comprises administering to the mammal an effective amount of a compound as claimed in any one of claims 1, 2, 7 or 14, a salt as claimed in any one of claims 3 to 6 or 14 or a composition as claimed in claim 15.

17. A method as claimed in claim 16 when used to treat cattle for mastitis.

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